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16. ABSTRACT

The lichens Pseudoparmelia caperata (L.) Hale, Usnea cf subfusca Stirt., and Umbilicaria mammulata(Ach.) Tuck. were exposed to simulated acidic rain with pH levels of 2.3, 3.0, 3.3, 4.3, or 5.6 and other ions in concentrations normally found in rain in the eastern United States. The pH levels of the most acidic treatments (3.3,3.0,2.3) were similar to those found in fog, cloud water, and occasional rainfall events. The pH 4.3 and 5.6 treatments compared to average ambient conditions in the eastern and western United States, respectively, and caused no significant effects on photosynthesis. However, after the first week of treatment, significant effects of rain pH at the most acidic treatments on gross photsynthesis were detected in P. caperata and U. mammulata, but not in U. cf subfusca. Visible effects of injury were also observed and included bleaching, necrotic spots, and necrotic margins, which resembled damage seen in field populations of U. mammulata, the most sensitive species.

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JOURNAL ARTICLE

EFFECTS OF SIMULATED ACIDIC RAIN ON ONE SPECIES EACH OF *PSEUDOPARMELIA, USNEA,* AND *UMBILICARIA*

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Abstract. The lichens *Pseudoparmelia caperata* (L.) Hale, *Usnea* cf *subfusca* Stirt., and *Umbilicaria mammulata* (Ach.) Tuck. were exposed to simulated acidic rain with pH levels of 2.3, 3.0, 3.3, 4.3, or 5.6 and other ions in concentrations normally found in rain in the eastern United States. The pH levels of the most acidic treatments (3.3, 3.0, 2.3) were similar to those found in fog, cloud water, and occasional rainfall events. The pH 4.3 and 5.6 treatments compared to average ambient conditions in the eastern and western United States, respectively, and caused no significant effects on photosynthesis. However, after the first week of treatment, significant effects of rain pH at the most acidic treatments on gross photysnthesis were detected in *P. caperata* and *U. mammulata*, but not in *U.* cf *subfusca*. Visible effects of injury were also observed and included bleaching, necrotic spots, and necrotic margins, which resembled damage seen in field populations of *U. mammulata*, the most sensitive species.

1. Introduction

Lichens obtain most of their nutrients for growth from the atmosphere and often respond to atmospheric contaminants that result from man's activities. They are known to be differentially sensitive to gaseous pollutants such as SO_2, NO_2, photochemical oxidants, and F (Anderson and Treshow, 1984).

During the last decade, acidic deposition has been recognized as an additional potential threat. For instance, mean rainfall acidity in the Ohio River Valley is about pH 4.2, with ranges of approximately 3.2 to 6 (US EPA, 1983). Cloud water in New Hampshire subalpine for forests has a mean H^+ concentration that corresponds to a pH of 3.5 (Lovett *et al.*, 1982); in southern California, the median acidity of fog samples taken during a span of more than 26 days corresponds to a pH less than 3 (Waldman, 1984).However, there is little evidence that acidic deposition affects lichens, but it is reasonable to hypothesize that lichens may be sensitive to this aqueous form of pollution because they are dependent on moisture in the atmosphere for metabolic activity. In addition, dissolution of dry particles (e.g., sulfates, nitrates) previously deposited on thalli is likely to enhance the effects of wet deposition.

Early studies of the effect of pH on lichens were done primarily with buffered aqueous solutions of S compounds (Puckett *et al.* 1973, 1974; Hill, 1971; Turk and Wirth, 1975; Baddeley *et al.*, 1971). In general, it was found that decreasing pH enhanced toxicity. Dennison *et al.* (1977) showed that N fixation decreased in *Lobaria* spp. as a function of decreased pH. However, Bailey and Larson (1982) found little effect on rates of CO_2 exchange in *U. mammulata* at pH levels as low as 2.0. In recent studies that have used more realistic methods of simulating natural rain, Lechowicz (1982) reported reduced photosynthesis in *Cladina stellaris* (Opiz.) Brodo at pH levels less than 4. Treatment of

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the same species in the field showed no significant effects on the growth rate of the lichen after 2 yr, but thallus discoloration was apparent below pH 3.5. After 3 yr, growth was significantly depressed in the sulfate-rich treatments below pH 4.5 (Lechowicz, 1984). Sigal and Johnston (1985) found that N fixation and photosynthesis were curtailed in *Lobaria pulmonaria* by exposure to simulated acidic rain with pH 2.6. Neither higher pH levels (4.2 and 5.6) nor O_3 (118, 235, 353, $\mu g\ O_3\ m^{-3}$; 1,960 $\mu g\ O_3\ m^{-3}$ = 1 ppm) and acidic rain combinations significantly affected photosynthesis or N fixation.

In the present study, *P. caperata*, *U.* cf *subfusca*, and *U. mammulata* were exposed to simulated acidic rain in the laboratory to determine (1) thresholds for effects on photosynthesis and (2) symptoms of tissue damage that might be related to field observations.

2. Materials and Methods

In March and October of 1981, *P. caperata* and *U.* cf. *subfusca* were collected from trees at the Oak Ridge National Environmental Research Park for duplicate simulated acidic rain experiments. In April, *U. mammulata* was collected from sandstone outcrops nearby and was included in the first experiment only. Each collection was stored in a growth chamber with an 11-hr photoperiod (115 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux density (PPFD)). Day and night temperatures were maintained at 20 and 10 °C, respectively. The lichens were wetted twice with distilled water during a 3-day acclimization period prior to treatment. Twice per week for 6 weeks, the lichens were exposed to simulated acidic rain (1.1 cm, 40 ± 5 min) with pH adjusted with H_2SO_4 and HNO_3 in a 1.5 : 1 molar ratio to 2.3, 3.0, 3.3, 4.3, or 5.6 on a greenhouse rain simulator (Shriner, 1978). Rain simulants were mixed to mimic the ionic concentrations normally found in rain in the eastern United States (Irving *et al.*, 1981). Between treatments, lichens were stored as described above and allowed to dry.

Each week, marginal tips of *U.* cf *subfusca* and *P. caperata* ($\sim 36\ mm^2$) and marginal plugs of *U. mammulata* ($\sim 28\ mm^2$) were exposed to a 30-s pulse of $^{14}CO_2$ in a porometer similar to that of Shimshi (1969). These exposures were conducted in a growth chamber at 20 °C and 315 $\mu mol\ m^{-2}\ s^{-1}$ PPFD. Immediately following exposure, samples were put in scintillation vials on dry ice to halt metabolic activity and then oven dried for 24 hr at 80 °C. After weighing, the samples were combusted in a Packard Tri-carb sample oxidizer, Model C 306 and the released $^{14}CO_2$ was trapped in a CO_2 absorbent (Carbo-sorb) and flushed into a collection vial with a ^{14}C scintillator (Permafluor V). Radioactivity in the samples was determined by standard liquid scintillation procedures on a Packard Tri-carb 460 C liquid scintillation counter. Calibration standards of known activity were oxidized and counted with each set of samples to monitor instrument efficiency. Results of this investigation are reported as gross photosynthesis in disintegrations per minute per milligram of lichen tissue ($dpm\ mg^{-1}$) for *Pseudoparmelia* and *Usnea* and dpm per plug ($\sim 28\ mm^2$) for *Umbilicaria*. Net photosynthesis was not calculated because photorespiration and mitochondrial respiration were not measured but the approximate values for *P. caperata* and *U.* cf *subfusca in* this study

range from 1.96 to 3.70 mg CO_2 kg^{-1} s^{-1} and for *U. mammulata*, from 0.02 to 0.05 mg CO_2 kg^{-1} s^{-1}. These values are reasonable for *Pseudoparmelia* (Link *et al.*, 1984) but are low for *Umbilicaria* (Larson, 1979). There were 8 replicates for each treatment per week for *P. caperata* and *U. subfusca* and five replicates for *U. mammulata*.

To describe and record color changes, thalli were compared at the beginning and end of the experiment to standard reference colors in the Munsell Color Charts for Plant Tissues (1977). All comparisons were made on dry lichens under sunlight at 2 : 00 p.m. ($\pm$ 1 hr) in the greenhouse.

Data were analyzed using the Statistical Analysis System (SAS, 1982) programs for the General Linear Models procedure. Significant differences between means were determined by tests of least significant difference (LSD). The analysis of variance (ANOVA) indicated no significant effects due to weak or weak-rain interaction; therefore, data from the individual weeks were pooled. Each point represents a sample size of approximately 80 for *P. caperata* and *U.* cf *subfusca* and 30 for *U. mammulata*.

3. Results and Discussion

The ANOVA indicated significant ($P \leq 0.05$) effects of simulated acidic rain on gross photosynthesis of *P. caperata* and *U. mammulata*, but not *U.* cf *subfusca*. The test for LSD indicated that the threshold for significant reduction of gross photosynthesis in response to treatment is between pH 3.0 and 2.3 for *P. caperata* (Figure 1). Although not statistically significant, the tendency for reduced gross photosynthesis at pH 2.3 was similar for *U.* cf *subfusca*. Gross photosynthesis of *U. mammulata*, in contrast to that of *P. caperata* and *U.* cf *subfusca*, was reduced approximately threefold at all of the pH levels from that at the average ambient-level treatment (pH 4.3). For these species, optimum photosynthesis in the laboratory was at or near pH levels similar to those in the ambient environment in which they grow. *Umbilicaria mammulata* was the most sensitive of the species tested; photosynthesis at the pH 5.6, 3.3, 3.0, and 2.3 treatments was 54 to 71% lower than that at the pH 4.3 treatment. *Pseudoparmelia caperata* was least sensitive, showing reduced (29%) photosynthesis only at the pH 2.3 level compared to that at the pH 4.3 and 5.6 treatments and slight enhancement (10 to 16%) at the 3.3 and 3.0 levels. At the pH 2.3 level, photosynthesis in *U.* cf *subfusca* was reduced 65% from that at the pH 4.3 treatment and 8 to 17% from that at the other treatment levels. The variability of the photosynthesis data was high for this fruticose lichen and the differences were not significant; however, a trend for decreased photosynthesis is seen at the pH 3.0 and 2.3 treatments. Photosynthetic variability in fruticose lichens was also noted by Ross and Nash (1983) and Lechowicz (1982). Thus, sample size for multi-branched species may need to be large to determine significance of effects.

Significant differences were found between the repeat experiments for *P. caperata* (Table I), which may be due to seasonal variations, but the treatment effects were similar for each experiment. For *U.* cf *subfusca*, no significant differences were observed between repeats which suggests that laboratory conditions were either not responsible for the differences in the *P. caperata* repeat or that *P. caperata* is more sensitive to

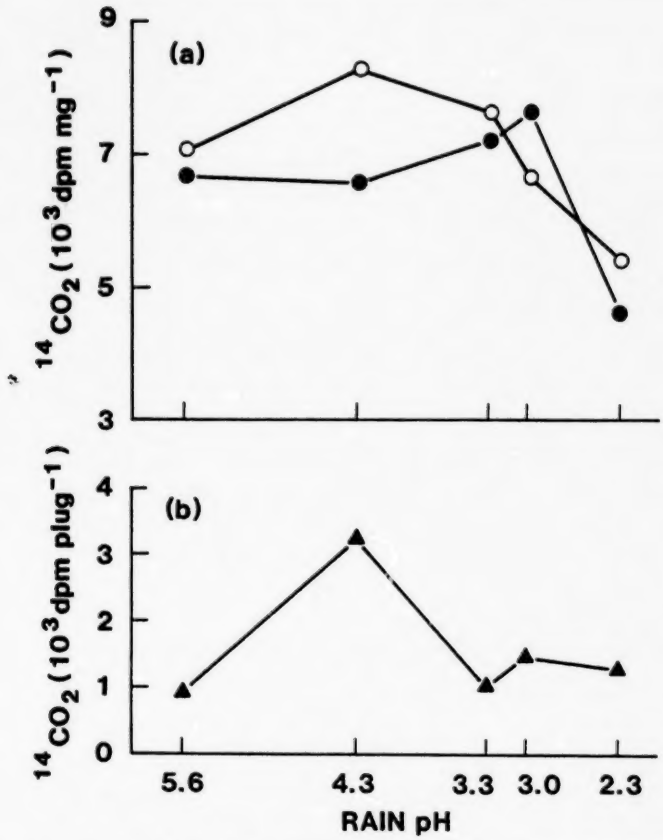

Fig. 1. Gross photosynthesis of lichens in response to simulated acidic rain; (A) data for *Pseudoparmelia caperata* (●) and *Usnea* cf *subfusca* (O) are dpm per mg and (B) data for *Umbilicaria mammulata* (▲) are dpm per plug (~28 mm^2).

TABLE I

Elemental concentration of plugs (~28 mm^2) of necrotic and nonnecrotic portions of the field-collected thallus of *Umbilicaria mammulata* shown in Figure 2; means and standard errors for a sample size of two

	Element concentration, μg g^{-1} dry wt			
	Al	Fe	K	Mg
Uninjured	535 (5)	220 (10)	5900 (101)	510 (10)
Necrotic	1800 (303)	415 (25)	4400 (101)	390 (41)

environmental conditions. Although the *U. mammulata* experiment was not repeated, the weekly results consistently showed a similar pattern. The response to treatment for all three species occurred after the first week and did not vary significantly over the 6-week study; therefore, short-term experiments of 1 to 2 weeks are adequate under our laboratory conditions to determine short-term effects of simulated acidic rainfall on gross photosynthesis. The relationship of these effects to effects of ambient acidic precipitation episodes on growth and survival of these species in the field is unknown. However, Lechowicz (1984) shows that many artificial episodes of simulated acidic rain in the field are necessary before growth is depressed in *Cladina stellaris*.

In addition to decreased photosynthesis in *U. mammulata*, distinct necrotic margins (about 1 mm wide) developed after two treatments at pH 2.3. This response was unique to the acidic rain treatment because, in another study (Sigal, unpublished data), *U mammulata* was exposed to SO_2 and O_3, alone and in combination, and no visible symptoms of tissue damage were observed. The necrotic margins produced in the laboratory were reminiscent of injury seen in field populations of *U. mammulata* collected in Tenessee (Figure 2). Chemical analysis by inductively coupled plasma spectrophotometry (ICP; Baes *et al.* 1983) of the field-collected thallus showed that the necrotic areas contained higher levels of heavy metals, notably Al and Fe, and lower levels of essential elements, notably K and Mg, compared to uninjured portions of the thallus (Table I). These data are similar to those from studies of trace elements in tree rings in which it is suggested that increases in trace metal availability, in particular Fe and Al, may result from acidic precipitation (Baes and McLaughlin, 1984). A decrease in tree growth (Puckett, 1982) and the decline of umbilicate lichens, including *U. mammulata*, (Smiley and George, 1974) in the Lake Mohonk area of the Shawangunk Mountains (about 130 km north of New York City) has been documented. The authors suggested that precipitation associated with winds contaminated with stack exhausts from a local power plant, in addition to other regional pollution, may have contributed to the decline. Based on the results of our study, we can hypothesize that acidic depostition may be a factor in the decline of these lichen species.

For *P. caperata* and *U.* cf *subfusca*, the color of the lichen thalli changed as a result of all of the treatments (Table II). The thalli of *P. caperata* bleached from the normal gray-green lichen color (7.5. GY 8/2) to a more yellowish color (2.5 GY 8/4) as a result of the pH 5.6 and 2.3 treatments. An intermediate color (5.0 GY 8/2) was recorded for the pH 4.3, 3.3, and 3.0 treatments. In addition, necrotic spots developed on the *P. caperata* thalli as a result of the pH 3.3, 3.0, and 2.3 treatments. For *U.* cf *subfusca*, bleaching from the normal gray-green lichen color (5.0 GY 6/2) resulted from all treatments, but the yellowish cast of the bleached thalli was most pronounced at the treatments with the highest acidity (i.e., pH 3.3, 3.0, 2.3); these same lichens also showed a slight reddish brown tinge when the thalli were wet.

For *U.* cf *subfusca* and *P. caperata*, thallus bleaching was visible after 6 weeks of treatment, but bleaching was not specific to the high acid treatments and thus did not reflect the trend for decreased photosynthesis. Moreover, thallus bleaching is also symptomatic of O_3 (Sigal and Nash, 1983) and SO_2 injury (Showman, 1975). However,

Fig. 2. *Umbilicaria mammulata* showing necrotic rings that may be the result of ambient acidic deposition.

for *P. caperata*, necrotic spots developed at the pH 3.3, 3.0, and 2.3 treatments. Necrotic spots also develop on tomato (Bisessar *et al.*, 1984) and birch leaves (Wood and Bormann, 1974) from low pH rain treatments. Thus, necrotic spots or margins on lichen thalli may be symptomatic of injury from acidic deposition as we are unaware of any reports of necrotic areas on lichens resulting from experiments with gaseous air pollutants.

TABLE II

Changes in lichen thallus color as represented by Munsell Color Charts after 6 weeks of simulated acidic rain treatments [a]

Treatment (pH)	*Pseudoparmelia caperata*	*Usnea* cf *subfusca*
No treatment	7.5 GY 8/2	5.0 GY 6/2
5.6	2.5 GY 8/4	2.5 GY 7/2
4.3	5.0 GY 8/2	2.5 GY 7/2
3.3	5.0 GY 8/2	2.5 GY 7/4
3.0	5.0 GY 8/2	2.5 GY 7/2 to 7/4
2.3	2.5 GY 8/4	2.5 GY 7/2 to 7/4

[a] The numerical representation for color is based on standard colors as provided by Munsell Color Charts (1977).

The levels of acidity in the simulated rain used in this study represent a range of concentrations spanning unpolluted through very polluted scenarios. The pH 5.6 and 4.3 levels are similar to those typical of current ambient average conditions in the western and eastern United States, respectively (US EPA, 1983); the 3.3 level is representative of low pH rain episodes in the eastern United States (US EPA, 1983) and is similar to the mean H^+ content in cloud water in New Hampshire (Lovett *et al.*, 1982); and the 3.0 and 2.3 levels are representative of the acidity found in fog water in southern California (Waldman, 1984).

We concluded that the lichens tested are not being affected by average ambient rainfall conditions in the eastern United States. However, averages hide extreme pH values of rainfall episodes and our studies show that two simulated low pH events (i.e., 1 week of treatment) are enough to affect gross photosynthesis in the species tested. Thus, the frequency of ambient, low pH rainfall episodes or the occurrence of low pH fogs and mists may be important to lichen vitality and subsequently to species richness and abundance.

Neither our study nor those of others have considered the impact of solubilized dry deposition because of the difficulty of simulating dry deposition of the small (≤ 2 μm) particles of importance (i.e. sulfate, nitrate, ammonium) in the laboratory. However, wet and dry deposition processes are of about equal importance in the average deposition of specific chemical species (US EPA, 1983). Thus the impact of acidity on lichens may be greater under ambient conditions than under laboratory conditions where the effects of solubilized, accumulated dry deposition are absent.

Based on this study and those of Sigal and Johnston (1985) and Lechowicz (1982, 1984), additional experiments are needed in the pH 2.6 to 4.0 range to determine a threshold for effect. These studies should incorporate varying ratios of the dominant sulfate-nitrate ions and include conditions that mimic acidic fog or cloud water.

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